

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61L 27/00, 25/00, A61K 9/00		A1	(11) International Publication Number: WO 96/10428 (43) International Publication Date: 11 April 1996 (11.04.96)
(21) International Application Number: PCT/US95/12802 (22) International Filing Date: 3 October 1995 (03.10.95) (30) Priority Data: PCT/US94/11209 3 October 1994 (03.10.94) US		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(60) Parent Application or Grant (63) Related by Continuation US PCT/US94/11209 (CIP) Filed on 3 October 1994 (03.10.94) (71) Applicant (for all designated States except US): OTOGEN CORPORATION [US/US]; 2454 Embarcadero Way, Palo Alto, CA 94303 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): SIERRA, David, H. [US/US]; 48 Middle Gate, Atherton, CA 94027 (US). (74) Agent: KONSKI, Antoinette, F.; Morrison & Foerster, 755 Page Mill Road, Palo Alto, CA 94304-1018 (US).			
(54) Title: DIFFERENTIALLY BIODEGRADABLE BIOMEDICAL IMPLANTS (57) Abstract <p>The current invention is a biomedical implant comprising a biomedical matrix material and a biodegradable porosifying agent. As the porosifying agent degrades <i>in situ</i>, an implant with an interconnecting network is formed. The resultant mechanically stable implant allows for tissue and fluid influx into the matrix. The invention is also directed to a method for repair of mammalian tissue using the above-described implant.</p>			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

5

DIFFERENTIALLY BIODEGRADABLE BIOMEDICAL IMPLANTS

This application is a continuation-in-part of U.S. Serial No. 08/522,299, filed October 3, 1994, the contents of which are hereby incorporated by reference 10 into the present disclosure.

DescriptionTechnical Field

This invention is in the general field of 15 biomaterials. More specifically, the invention is directed to biomedical implants, their composition and methods of preparation and use.

Background of the Invention

20 Biomaterials have been used for implantation into the human body to act as supports for wound and solid tissue healing. Matrices useful for this purpose should have the ability to adhere and conform to the wound site and surrounding tissue. Ideally, they also 25 should facilitate accumulation of fibroblasts, endothelial cells and wound healing regulatory cells to promote connective tissue deposition and angiogenesis.

U.S. Patent No. 4,849,285 to Dillon is directed to a composite, self-supporting agglomerated 30 macrostructure useful as a surgical implant. The macrostructure is a matrix of polytetrafluoroethylene resin and cured silicone that has uniformly distributed within it a particulate material. These particulates have a maximum size of about 2000 microns and may be 35 hydroxyapatite or tricalcium phosphate. This particular

-2-

macrostructure, therefore, is a composite of ceramic particulate material and organic biomaterials that is uniformly permeated by a network of open pores. The pores are formed by incorporating sodium chloride into 5 the composite and thereafter leaching it out in the manufacturing process.

U.S. Patent No. 4,843,112 to Gerhart et al. is to a bone cement composed of a particulate biocompatible calcium phosphate ceramic and a resorbable calcium salt disperses in a crosslinked biodegradable polyester 10 matrix. Pores are created in the matrix by body fluids creating small voids or cavities in the polymer matrix.

U.S. Patent No. 5,141,522 to Landi et al. describes a composite of two or more biocompatible 15 polymers useful for mammalian tissue repair. One of the polymers is polytetrafluoroethylene (PTFE), which is the reinforcing binder. A bioabsorbable component that may be a lactone, carbonate or a lactide, is contained within the structure of the PTFE and serves to enhance ingrowth 20 of tissue.

Additional disclosures of PTFE compositions useful as implants include, but are not limited to U.S. Patent Nos. 5,141,522; 5,098,779; and 4,863,974. The PTFE component of these compositions serves as a 25 nonabsorbable microfibrillar structural support. A bioabsorbable component is contained or coated on the structural support. The PTFE is polymerized prior to implantation of the compositions.

U.S. Patent No. 4,373,217 to Draenert is 30 directed to a polymeric implant material that has an acrylate, polymethacrylate or copolymer base with dispersed resorbable tricalcium phosphate of 50 to 300 microns with an available pore volume of less than 0.1 mL/g. This particular material is said to allow for a 35 firm bond between implant and body tissue. Resorption of

- 3 -

tricalcium phosphate particles at the surface of the implant are resorbed into the body is said to promote bone growth in the marginal porosity produced. In order to ensure absorption of liquid monomer into the porous 5 calcium phosphate, a filler that is also resorbable in the body is included to fill the pore volumes of the calcium phosphate.

U.S. Patent No. 4,898,734 to Mathiowitz et al. also involves a precast solid polymeric implant material. 10 A continuous polymeric matrix made of, for example, polyurethane or polystyrene, is embedded with microcapsules or microspheres that may contain material for subsequent release. The spheres may be removed from the matrix by bioerosion. For creation of a vascular 15 graft, erodible microspheres are entrapped within a tube-shaped slower-degrading polymer matrix. Rapid erosion of the spheres results in pores for cell seeding and vascularization with the matrix providing support until there is sufficient cell growth to create structural 20 integrity.

U.S. Patent No. 4,950,483 to Ksander et al. describes a collagen implant useful for wound healing. The implant is made of collagen and has a bulk density of 0.01 to 0.03 g/cm³ and is said to have a pore size 25 sufficient to permit cell ingrowth. Bioactive agents such as FGF and TGF- β may be incorporated into the implant.

U.S. Patent No. 5,077,049 to Dunn et al. is directed to a method for restoring periodontal tissue. A 30 biodegradable liquid polymeric systems designed to generate a porous structure when cured into a barrier membrane, is administered to the soft-tissue defect. The pores will form as a result of water-soluble material included in the liquid material. The liquid material 35 injected into the defect provides a scaffold that is

-4-

filled with new bone cells that gradually replace the water-soluble polymer.

U. S. Patent No. 4,902,295 to Walthall et al. involves a transplantable artificial tissue. The tissue 5 is made by mixing a polymerizing matrix with reversible gel precursors in an aqueous solution with viable cells. The gel, which may be alginate, a gum or agarose, is then dissolved to provide a porous matrix for implantation.

None of the above-described references 10 describes a biomedical implant material with a differentially degradable matrix and porosifying agent where polymerization occurs *in situ* or where the matrix is precast and is made of a biopolymeric material.

15 Disclosure of the Invention

Accordingly, one aspect of the present invention is an *in situ* polymerizing biomedical implant useful for implantation into a patient comprising a slowly biodegradable matrix material and a biodegradable 20 porosifying agent.

Another aspect of the invention is a precast biomedical implant useful for implantation into a patient comprising a slowly biodegradable polymeric matrix or a nonbiodegradable ceramic matrix and a biodegradable 25 porosifying agent.

A further aspect of the invention is a method for repair of mammalian tissue using the above-described implants.

Modes of Carrying Out the InventionDefinitions

As used herein, certain terms will be used which have defined meanings.

5 By "biodegradable" or "bioerodible" as it relates to the porosifying agent is intended a material that will dissolve *in situ* as a result of exposure to an aqueous environment in less than a week, preferably about 1 and 72 hours, more preferably between about 2 and 12
10 hours. Dissolution may occur as a result of a number of different mechanisms such as simple diffusion, hydrolysis, enzymatic cleavage, ion exchange, autocatalysis, osmosis, degradation, free-radical cleavage, radiation effect, thermal melting, and chemical
15 dissolution. Hydrolysis is the preferred mechanism for biodegradation. As such, the biodegradation of the porosifying material is distinguishable from prior art "leaching" of water-soluble drugs and salts, such as particulate calcium salts, e.g., tricalcium phosphate.
20 Typically, these water-soluble drugs or salts merely create small voids or cavities on the surface of the matrix in contrast to the porous network provided by the biodegradable porosifying agents described herein.

By "slowly biodegradable" or "slowly
25 bioerodible" as it relates to the matrix material is intended a material that will not dissolve *in situ* (or in an aqueous environment) within a week, or may dissolve in a period of from about one week to 24 months, preferably a period of between about 1 to 12 months. It also is
30 intended to exclude material such as a polyether that is only degradable outside the range of normal body temperature and in organic solvents. Examples of this type of excluded polyether include low molecular weight aliphatic polyethers which are soluble in aqueous
35 solutions of methanol, ethanol or acetone.

-6-

The term "porosifying agent" intends particulate materials that include but are not limited to materials in the form of solid or hollow spheres, extruded rods, or other convenient shapes. Typically, 5 the particulate has a mean diameter of between about 10 and 500 μm , more typically between about 20 and 200 μm . The particles are generally spherical in shape but other shapes such as rhombic, irregular, stellate and other crystalline type shapes may be used. The agents are 10 present in a concentration of at least about 12% per volume of the matrix material, preferably the concentration is between about 12 and 99% per volume of the matrix material, more preferably between about 20 and 90% per volume of the matrix material such that as the 15 agent biodegrades a continuous porous network or pathway is formed within the implant. In one embodiment, components such as calcium salts, alginate, gum or agarose are specifically excluded.

The term "matrix" intends the portion of the 20 implant material that acts as the support network; it is the slower biodegrading portion of the implant.

The term "continuous porous network" is intended to describe a network of micro-spacings or an internal micro-network formed by the biodegradation of 25 the porosifying agent. The micropores are internally and externally interconnected to form a tunnel-like system or network within and throughout the matrix.

The Implant Material

30 This invention is to a biomedical implant comprising a non-toxic, slowly biodegradable biomedical matrix material and a non-toxic, biodegradable material that acts as a porosifying agent. The porosifying agent is present in sufficient quantity and particulate size to

- 7 -

result in a continuous, porous network within the matrix once it has degraded.

5 The implant is biocompatible and is capable of solidifying when being cast or of solidifying and polymerizing *in situ*. Further, the matrix is slowly biodegradable as defined above and made from a material with a slower degradation rate than the porosifying agent. Degradation (or dissolution) rates of particular substances in water are generally available information.

10 Examples of matrix materials include but are not limited to collagen, fibrin, fibrinogen, polyorthoesters, polyvinyl alcohol, polyamides, polycarbonates, polyvinyl pyrrolidone, marine adhesive proteins, cyanoacrylates, analogs (e.g., fibrinogen

CHANGED

15 analog as PCT WO 94/16085 to Irani for "Hybrid proteins having crosslinking and tissue binding activities", the contents incorporated herein by reference) mixtures, combinations and derivatives (e.g., methylated collagen, pegulated marine adhesives proteins) of the above.

20 Preferred mixtures of the above for the matrix is a fibrin/collagen matrix in combination with gelatin as the porosifying agent. In one embodiment, polytetrafluoroethylene (PTFE), calcium phosphate ceramics, and materials that are not amenable to 25 polymerization *in situ* such as polyethylene are specifically excluded as matrix materials.

113

The porosifying agent is biocompatible and biodegradable as described above. Examples of porosifying agents include but are not limited to 30 gelatin, gelatinized collagen, collagen, fibrin, fibrinogen, proteins in solid state like albumin powder, degradable polyesters (polylactic or polyglycolic acid), polyethylene glycol (PEG), liposomes, lipids with emulsifiers, alginates, analogs, mixtures, combinations 35 and derivatives of the above. Preferred mixtures of

-8-

porosifying agents include pegulated particulates, albumin microspheres and gelatin. The porosifying agents may be in a solid state, such that they dissolve over a period of time or may they may be altered such that they are in a sparingly soluble state. This may be accomplished for example by altering the pI, for example by methylation or succinylation or by conjugating the porosifying agent to polyethylene glycol (MW 1 to 50 Kd) or by crosslinking said with glutaraldehyde.

10 In one embodiment, the matrix material is biodegradable but at a rate which is slower than the porosifying agent. Preferably, the matrix material is any of collagen, a collagen analog, e.g., gelatinized collagen, fibrinogen, or functional equivalents thereof.

15 As such, materials such as PTFE and bone substitutes as described above are specifically excluded in that these materials are nonabsorbable yet biocompatible materials. Additionally, PTFE is not capable of polymerization *in situ*. When the matrix is composed of collagen, the

20 collagen is preferably not chemically crosslinked, although it can be if desired.

In addition to the matrix material and porosifying agent, the implants may further include growth factors including but not limited to epidermal growth factor (EGF), transforming growth factor β (TGF β -1, TGF β -2), platelet derived growth factor (PDGF-AA, PDGF-AB, PDGF-BB), fibroblast growth factor (FGF), insulin-like growth factors (IGF), tumor necrosis factors (TNF), colony stimulating factors (CSFs), nerve growth factors (NGF), brain-derived neurotropic factor (BDNF). (Amgen, Thousand Oaks, CA and Regeneron, Inc. Tarrytown, NY), ciliary neurotropic factor (CNTF) (Amgen, Thousand Oaks, CA and Regeneron, Inc. Tarrytown, NY) and the like, and/or therapeutic agents including but not limited to 30 cytokines, interleukins (IL-1, IL-2) or other co-factors

- 9 -

such as heparin or calmodulin, antibiotics, antineoplastic and antibacterials, to further stimulate or control tissue remodeling, or to control sepsis. These agents can be incorporated into the matrix 5 material, the porosifying agent, or both. When the therapeutic is incorporated into the porosifying agent, it is released at rate greater than the matrix material.

An important characteristic of the implant is that the porosifying agent degrades faster than the 10 matrix material. For example, if fibrin is used as the matrix, then polyethylene glycol or gelatin, which degrade more rapidly in water (and thus *in situ*) than does fibrin, may be used as the porosifying agent. However, if fibrin is used as the porosifying agent, then 15 collagen may be used as the matrix since it degrades more slowly than does fibrin.

Preparation of the Implant
in situ Polymerizing Systems

20 In an *in situ* polymerization system, the porosifying agent may mixed as a dry phase with the matrix which may be in a semi-solid, liquid or dry particulate phase. An appropriate catalyst or co-factor may be added to the mixture or the porosifying agent 25 itself may contain such catalyst or co-factor that will initiate polymerization.

Fibrin sealants are an example of an *in situ* 30 polymerizing system. Fibrin sealants are two component tissue adhesive systems that are in a relatively viscous liquid form until both components are mixed together and polymerize at the surgical application site into a relatively dense gel. Thrombin in combination with Ca^{2+} catalyzes the polymerization of fibrinogen, converting the fibrinogen into fibrin polymer. Further, thrombin 35 and Ca^{2+} activate coagulation Factor XIII, which effects

-10-

covalent crosslinking of fibrin. The rate of proteolytic degradation of the fibrin polymer clot is decreased and mechanical stability is increased as a result of the covalent crosslinking of the polymer.

5 The fibrin polymer clot is porous, but only at a range of 1 to 5 micron in mean diameter, too small to permit cellular ingrowth. Accordingly, macrophage activity is sustained over periods of time longer than optimal for degradation and remodeling, and the fibrin

10 polymer clot acts as a barrier until phagocytosis is complete. Where a porosifying agent is added according to the present invention, tissue reunion is improved as a result of the continuous pathway formed in the clot when the porosifying agent degrades *in situ*.

15 For systems where the matrix is made of fibrin, particulates may be incorporated directly into the fibrinogen component which is obtained in lyophilized form. The particulates may be alginate, gelatin, polyethylene glycol, polylactic acid/polyglycolic acid

20 (PLA/PGA) hollow spheres, lipid in an emulsifier system (e.g., lecithin, Triton, lauryl sulfate, or Tween-80) hyaluronic acid and liposomes or other materials that degrade at a rate faster than the fibrin matrix and will create a continuous porous network once degraded. The

25 porosifiers may be incorporated either in dry or liquid or semisolid form. Alternatively, the porosifier may be mixed just prior to, or during application of the system to the repair site. In another embodiment, collagen can be used as the matrix material of the inventive implants.

30 When collagen is the matrix material, the porosifying agent may preferably contain an effective fibrin-forming amount of thromboplastin (Ortho Diagnostic, Raritan, N.J.). These composites are particularly useful for tissue repair or effecting hemostasis by administering to

35 the wound or treatment site a therapeutic amount of the

-11-

composite. The particulate is preferably a hydrophilic porosifier such as gelatin, gelatinized collagen, fibrin, a salt or polyethylene glycol.

5 Pre-cast Systems

Alternatively, the matrix with the porosifying agent may be preformed and used for surgical reconstruction and drug delivery. In a particular embodiment, the implant is applied to the wound site as a dressing. The matrix 10 material may be fibrin, alginate, collagen, PLA/PGA or other biocompatible polymers as well as rapidly dissolving ceramic based systems such as calcium sulfates, calcium phosphates, and the like. Porosifying agents such as gelatin, fibrin, polyethylene glycol are 15 added to the matrix material. Exudate from the repair site anchors the dressing in place by infiltrating the porous network produced as a result of the degrading particulate. Tissue adhesives including but not limited to materials such as fibrin sealants and occlusive wraps 20 and tapes may help to anchor the dressings in place.

Use of the Implant

When the implant is placed or applied to a desired site *in vivo*, the porosifying agent biodegrades 25 relatively rapidly, thus leaving behind an inter-connecting network of pores to permit tissue and fluid influx into the matrix. The matrix then acts as a scaffolding for the migrating cells (e.g. macrophages, fibroblasts, and neovascular endothelial cells) and will 30 degrade as these cells express connective tissue components for remodeling and regeneration.

The use of a matrix with a component that degrades *in situ* imparts several advantages over conventional porous implant configurations. First, 35 porous implants tend to shrink in volume due to pressure

-12-

from surrounding tissue, thus minimizing the benefits of controlled pore size and minimizing the amount of tissue ingrowth that can take place. Where a porosifying agent that degrades *in situ* is added, however, the cells 5 involved in wound healing migrate into the network and minimize shrinkage of the implant.

A further benefit of an *in situ* degrading porosifying agent is that the porosifying agent acts as a mechanical stabilizer, permitting the formation of a 10 porous network within the matrix. Materials such as gelatin, especially crosslinked gelatin, calcium alginate or fibrin are especially useful as the porosifying agent. Crosslinking may be accomplished by the addition of agents such as SPEG (polyethylene glycol succinimydyl), 15 glutaraldehyde, diisocyanate, or dehydrothermally. Where calcium alginate is the porosifying agent, the guluronic/mannuronic acid segment ratio may be optimized for *in vivo* dissolution over the targeted period of time. Where fibrin is the porosifying agent, a high quantity of 20 plasmin (≈ 0.2 mg/mL) is also useful, permitting a degradation rate proportional to the quantity of plasminogen present. Where polyethylene glycol particulate is used as the porosifying agent, a relatively rapid dissolution occurs (i.e. in less than 24 25 hours).

Another benefit derived from using an *in situ* biodegradable porosifying agent is that the mechanical properties of the implant both pre- and post-polymerization can be altered, tailoring the viscosity of 30 the applied material and improving its mechanical stability *in situ*. The porosifying agent increases the stiffness modulus of the implant while it is still relatively undissolved. As dissolution occurs the contribution to the modulus by the porosifying agent 35 decreases. Deposited ground substances (i.e.

-13-

mucopolysaccharides, glycosaminoglycans, nectins and other proteoglycans) and collagen and inflammatory cells are exchanged, thus the overall modulus status remains roughly the same throughout the life-span of the matrix.

5 The rate of degradation of the implant materials will vary depending upon the material used (PEG the fastest, crosslinked gelatin the slowest) as well as the relative vascularity of the application site (liver, the fastest, subcutaneous, the slowest). A fibrin matrix
10 will last usually from 5 to 14 days, depending upon concentration, plasminogen content and anatomic region. Higher fibrin and lower plasmin concentrations will decrease degradation rates. The addition of antiproteases such as ϵ -amino-n-caproic acid or aprotinin
15 will retard degradation further. Once the implant is applied to the wound site, the porosifying agent begins to dissolve. This may occur in a matter of hours if the agent is polyethylene glycol or a matter of days if calcium alginate. The resultant porosity permits firm
20 anchoring to the wound bed by host fibrin clots intercalating through the porous network. Leukocytes, macrophages, lymphocytes and fibroblasts then migrate through the pores, breaking down the fibrin implant matrix and initiating deposition of ground tissue
25 substances (e.g. proteoglycans) and collagen. By way of example, implants tailored to last for 7 to 14 days may be applied to donor graft beds, chronic decubitus ulcers, resected tumor sites or bone tissue gaps.

30 In situ polymerizing systems are introduced into the repair site by a variety of means. They may be poured onto the site directly or by a dispenser which permits control of the amount of material in the system, as well as the area covered. The implants may be used as occlusive or fluid tight dressings or sealants in
35 anatomic regions where it would be difficult to use a

-14-

precast dressing, such as in endoscopic procedures. An example of a dispensing device is the DUPLOJECT® fibrin sealant delivery device (Immuno AG, Vienna, Austria).

Precast systems may be used as occlusive dressings. They are ultimately integrated into the repair site and facilitate tissue remodelling. In one embodiment, the precast systems contain an effective amount of thromboplastin as defined above. They may be suture, stapled, taped or wrapped into place. Generally, they are used as burn dressings or in tumor resection sites to facilitate reepithelialization. Also, they may deliver growth factors or antimicrobials (e.g., gentamicin, penicillin, silver ions) or other metabolic modulators (e.g. calcitonin, interleukins).

It is apparent to those skilled in the art that the compositions described herein are useful for the preparation of medicaments for any suitable use, for example, tissue repair or for the release of therapeutic agents.

It is to be understood that while the invention has been described in conjunction with the preferred specific embodiments thereof, that the foregoing description as well as the examples that follow are intended to illustrate and not limit the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the biomedical implant art.

Examples

30 Example 1 - Preparation of an *in situ* Polymerizing Fibrin Implant

In a tuberculin syringe with a 20 gauge needle, concentrated fibrinogen-Factor XIII (60mg/mL) in tris-buffered saline (pH 7.2) is mixed with polyethylene

-15-

glycol particulate (10000 MW, mean diameter 150 μm) to 50 vol%.

Example 2 - Preparation of an *in situ* Polymerizing

5 Calcium Alginate Implant

Calcium alginate microspheres (mean diameter 100 μm) prepared as described in Gospodarowicz and Cheng J. Cell Physiol 128:475-484 (1986) which is herein incorporated by reference in its entirety. These are 10 added in a syringe to 50 vol% as described in Example 1.

Example 3 - Preparation of an *in situ* Polymerizing
Gelatin Implant

SPEG-crosslinked gelatin

15 5 mL of concentrated collagen slurry in phosphate buffered saline (pH 7.2, 35 mg/mL, Zyderm I, Collagen Corp, Palo Alto, CA) is heated to 60°C for 1 hour in a water bath, and then chilled to 37°C to produce gelatin. Phosphate-buffered saline is added to dilute 20 the gelatin concentration to 15 mg/mL. Sufficient SPEG is added to the gelatin solution for a final concentration of 10 mg/mL. The gelatin-SPEG solution is allowed to cool to room temperature and gel. The gel is lyophilized and pulverized by a grinding mill. The 25 powder is sieved and particles in the range of 20 to 150 μm mean diameter are saved and sterilized by electron beam irradiation (2.5 Mrad dose).

The Matrix

30 The lyophilized SPEG crosslinked gelatin particulate is mixed with lyophilized fibrinogen-Factor XIII in a 1:1 v/v ratio. The powdered mixture is loaded into a dual plunger syringe system, containing both the lyophilate and the reconstituting buffer, Tris buffered 35 saline (TBS). To reconstitute the gelatin-fibrinogen

-16-

mixture, the plunger is depressed, forcing the diluent into the chamber containing the lyophilate. After several minutes incubation, the resultant slurry is ready to use.

5

Example 4- Preparation of a Pre-cast Fibrin Sealant System

12 vol % of polyethylene glycol particulate (MW 5kd with a mean diameter of 20 to 100 μm) is mixed with 10 the fibrinogen solution at a concentration of 30 mg/mL. The mixture is then poured into a mold. Polymerization of the fibrinogen is catalyzed by the addition of thrombin and Ca^{2+} , usually in a 1:1 v/v ratio. The catalyst is added rapidly and is thoroughly mixed to 15 prevent settling of the particulate. 10 U/mL of thrombin is added for rapid polymerization. After gelling, the implant may be stored refrigerated (2-10°C) or frozen (-20 to -150°) until ready to use.

20

Example 5 - Preparation of a Pre-cast Calcium Alginate System

Calcium alginate containing a minimum of 30% guluronic acid segments at 15 mg/mL in an aqueous solution is poured into a shallow rectangular mold.

25

Gelatin is added as the porosifying agent at a concentration of 20 vol %. Concentrated calcium chloride solution is titrated rapidly into the alginate/gelatin mixture to a 0.1 M final concentration in the calcium alginate solution to effect gelling. Agitation of the 30 mixture is necessary to minimize swelling of the particulate. The implant is sterilized by quick exposure to a liquid bactericide (e.g. alcohol) and stored at temperatures as described above.

35

Modifications of the above-described modes for carrying out the invention that are obvious to those of

-17-

skill in the fields of chemistry, materials science, medicine and related fields are intended to be within the scope of the following claims.

5

10

15

20

25

30

35

-18-

Claims

I claim:

1. A differentially biodegradable biomedical implant useful for implantation into a patient comprising:
 - 5 a slowly biodegradable biocompatible matrix material; and
 - 10 a biodegradable biocompatible porosifying agent,

15 wherein polymerization and solidification of said implant occurs *in situ* and wherein, upon degradation of the porosifying agent, a continuous porous network is formed within the implant.
2. The implant of claim 1 wherein the porosifying agent is in particulate form.
3. The implant of claim 1 wherein the porosifying agent is selected from the group consisting of gelatin, gelatinized collagen, collagen, fibrin, fibrinogen, proteins, degradable polyesters, polyethylene glycols, antibiotics, silver compounds, encapsulated cytokines, liposomes and alginates, lipids with emulsifiers, analogs, mixtures and derivatives thereof.
4. The implant of claim 1 wherein the matrix is selected from the group consisting of gelatin, collagen, fibrin, fibrinogen, polyorthoesters, polyvinyl alcohol, polyurethanes, marine adhesive proteins, 30 cyanoacrylates, polyacrylates, analogs, mixtures and derivatives thereof.
5. The implant of claim 1 further comprising a growth factor.

-19-

6. The implant of claim 5 wherein the growth factor is selected from the group consisting of TGF β -1, TGF β -2, FGF, EGF, PDGF-AA, PDGF-AB, PDGF-BB, IGF, TNF, CSF, BDNF, CNTF, NGF, analogs, mixtures and derivatives 5 thereof.

7. The implant of claim 1 further comprising a therapeutic agent.

10 8. The implant of claim 7 wherein the therapeutic agent is selected from the group consisting of cytokines, interleukins, heparin, calmodulin, antibiotics, antineoplastics and antibacterials.

15 9. A differentially biodegradable biomedical implant useful for implantation into a patient comprising:

a slowly biodegradable fibrin matrix material;
and

20 a biodegradable polyethylene glycol porosifying agent,

wherein polymerization and solidification of said implant occurs *in situ* and where upon degradation of the polyethylene glycol, a continuous porous network is 25 formed within the implant.

10. A differentially biodegradable precast biomedical implant useful for implantation into a patient comprising:

30 a slowly biodegradable biocompatible matrix material comprising a biopolymeric or a ceramic material;
and

a biodegradable biocompatible porosifying agent,

-20-

wherein upon degradation of the porosifying agent, a continuous porous network is formed within the implant.

5 11. The implant of claim 10 wherein the porosifying agent is in particulate form.

10 12. The implant of claim 10 wherein the porosifying agent is selected from the group consisting of gelatin, collagen, fibrin, fibrinogen, proteins, degradable polyesters, antibiotics, silver compounds, encapsulated cytokines, liposomes and alginates.

15 13. The implant of claim 10 wherein the matrix is selected from the group consisting of gelatin, collagen, fibrin, fibrinogen, and calcium phosphates.

20 14. The implant of claim 10 further comprising a growth factor.

25 15. The implant of claim 14 wherein the growth factor is selected from the group consisting of TGF β -1, TGF β -2, FGF, EGF, PDGF-AA, PDGF-AB, PDGF-BB, IGF, TNF, CSF, BDNF, CNTF, NGF, analogs, mixtures and derivatives thereof.

16. The implant of claim 10 further comprising a therapeutic agent.

30 17. The implant of claim 16 wherein the therapeutic agent is selected from the group consisting of cytokines, interleukins, heparin, calmodulin, antibiotics, antineoplastics and antibacterials.

-21-

18. A method for repair of tissue comprising introducing a differentially biodegradable biomedical implant into the tissue repair site, said implant comprising:

5 a slowly biodegradable biocompatible matrix material; and
a biodegradable biocompatible porosifying agent;

10 wherein polymerization and solidification of said implant occurs in situ, and wherein, upon degradation of the porosifying agent, a continuous porous network is formed within the implant.

19. A method for repair of tissue comprising introducing a differentially biodegradable precast biomedical into the tissue repair site, said implant comprising:

15 a slowly biodegradable biocompatible matrix material comprising a biopolymeric or ceramic material;
20 and
a biodegradable biocompatible porosifying agent,

25 wherein upon degradation of the porosifying agent, a continuous porous network is formed within the implant.

20. A method for tissue hemostasis comprising introducing the implant of claim 1 or claim 9.

30 21. The method of claim 20, wherein the implant further comprises an effective amount of thromboplastin.

-22-

22. A method for the *in situ* delivery of a chemotherapeutic or therapeutic agent which comprises introducing the implant of claims 5, 7, 14 or 16.

5

10

15

20

25

30

35

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/12862A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L27/00 A61L25/00 A61K9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 539 751 (ATRIX LAB INC) 5 May 1993 see column 11, line 18 - line 37; claims ---	1-17
X	EP,A,0 560 014 (ATRIX LAB INC) 15 September 1993 see column 9, line 14 - line 55 see column 11, line 31 - line 57; claims ---	1-17
A	WO,A,90 03768 (SOUTHERN RES INST) 19 April 1990 see claims ---	1-17
A	EP,A,0 192 068 (DOW CHEMICAL CO) 27 August 1986 see claims ---	1-17
		-/-

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *&* document member of the same patent family

4

Date of the actual completion of the international search

8 February 1996

Date of mailing of the international search report

16.02.96

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

ESPINOSA, M

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/12802

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO,A,95 03011 (RICE UNIVERSITY) 2 February 1995 see page 6, line 27 - line 34; examples 1-7 -----	1-17

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 95/12802

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 18-22 because they relate to subject matter not required to be searched by this Authority, namely:
Method for treatment of the human or animal body by surgery or therapy.
2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 95/12802

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0539751	05-05-93	US-A-	5324519	28-06-94
		AU-B-	2605492	29-04-93
		CA-A-	2079831	29-04-93
		JP-A-	5305135	19-11-93
EP-A-0560014	15-09-93	AU-B-	3117493	16-09-93
		CA-A-	2091552	13-09-93
		JP-A-	6007423	18-01-94
WO-A-9003768	19-04-90	US-A-	4938763	03-07-90
		AU-B-	4501789	01-05-90
		AU-B-	5067793	17-02-94
		EP-A-	0436667	17-07-91
		IL-A-	91850	30-03-95
		IL-A-	107393	29-06-95
		JP-T-	4503163	11-06-92
		US-A-	5278201	11-01-94
		US-A-	5340849	23-08-94
		US-A-	5278202	11-01-94
EP-A-0192068	27-08-86	US-A-	4636526	13-01-87
		US-A-	4634720	06-01-87
		AU-B-	589393	12-10-89
		AU-B-	5266186	28-08-86
		CA-A-	1290881	15-10-91
		JP-A-	61193666	28-08-86
		US-A-	4842604	27-06-89
		US-A-	5007930	16-04-91
		US-A-	4698375	06-10-87
		US-A-	4661536	28-04-87
WO-A-9503011	02-02-95	AU-B-	7402894	20-02-95